Supporting Information available (SI) for

Calculating Radiation Exposures during Use of ^{14}C-labelled Nutrients, Food Components, and Biopharmaceuticals to Quantify Metabolic Behavior in Humans

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Note: Radiation exposure calculation sheets from ^{14}C-nutrients were available to the other attached Excel file (Supporting Information_Table2).

Supporting 2 Figures and 3 Tables

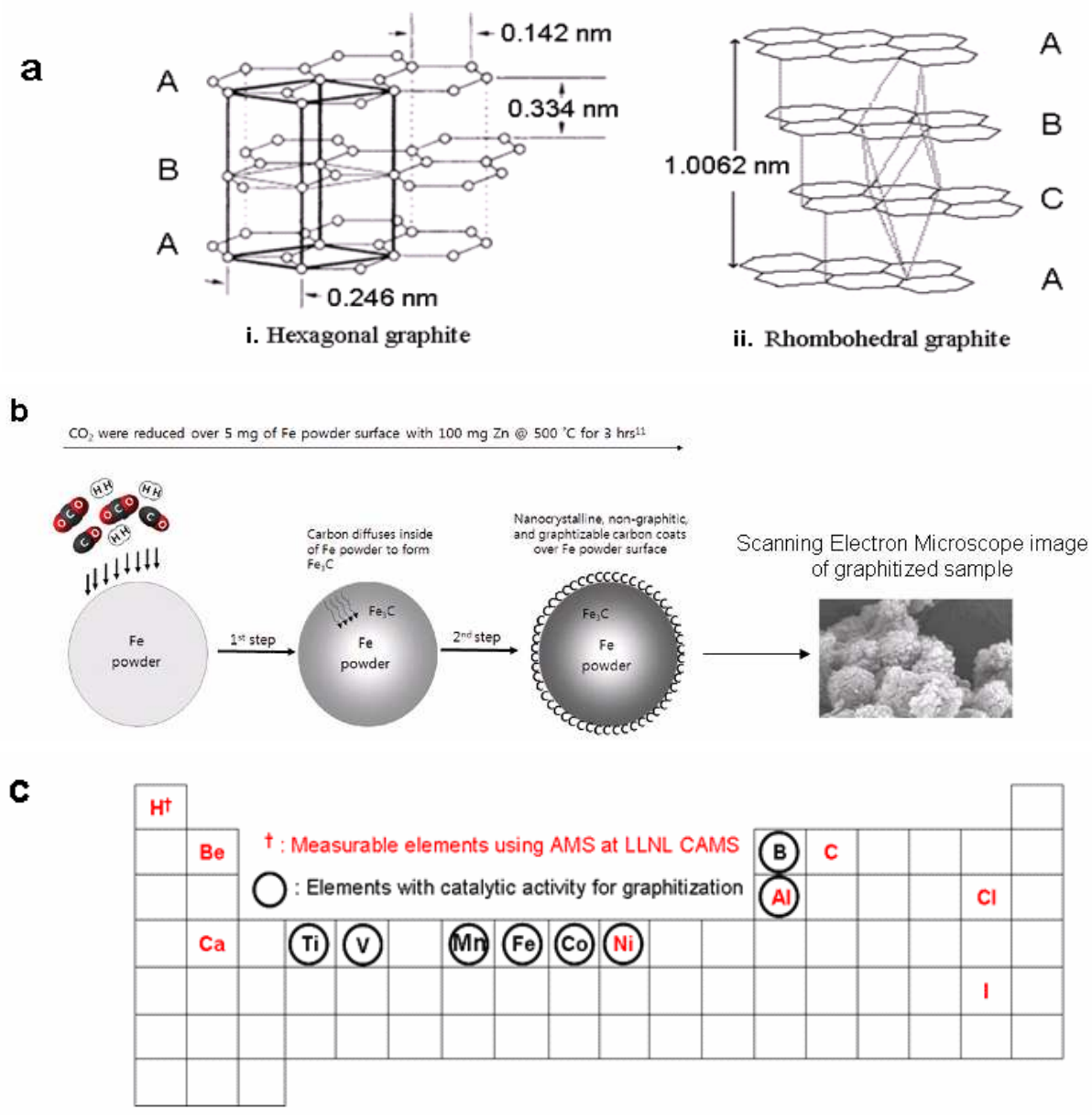

Figure 1. Graphite structure with hexagonal (i) or rhombohedral (ii) type (Figure 1a). A model of graphite or graphite-like materials formation was shown (Figure 1b). Elements in periodic

table (Figure 1c) have catalytic activity for graphitization (black circles) and were measured at CAMS LLNL (red color fonts) (*1-3*).

Graphitization for ^{14}C-AMS Applications. Graphite consists of multiple carbon hexagonal sheets (Figure 1a). Graphite is soft and grey/black in color. Graphite is classified as natural and synthetic forms with varying a variety of morphologies (*1, 4-5*). The natural abundance of graphite type is 70 % hexagonal (stacking sequence ABAB) and 30 % rhombohedral (stacking sequence ABCABC) (*5*). Graphite is the most important carbon in several industrial applications due to its thermal/chemical resistance as well as its high electrical conductivity (*1*).

For ^{14}C-AMS applications, all carbonaceous samples need to be converted to elemental carbon, graphite or graphite-like materials, over iron (Fe) or cobalt (Co) catalyst, that process is called graphitization. The graphitization process consists of oxidation (combustion: carbonaceous sample + CuO $\rightarrow$ CO_2, 900 °C, 2.5 h) and reduction (graphitization: CO_2 + Zn (/or H_2) + Fe (/or Co) $\rightarrow$ Graphite, 500 °C, 3 h) steps.

Figure 1b showed our suggested model of graphite or graphite-like materials formation during the reduction step (*6*). At first CO_2 was converted to iron carbides (especially, Fe_3C) that saturated the surface of the iron particle. Then, graphite or graphite-like materials were produced on the iron carbide surface. Finally, graphite or graphite-like materials over Fe or Co (Figure 1b) was packed into an AMS target holder for ^{14}C-AMS measurement.

Graphite or graphite-like materials have been viewed as the best material type for ^{14}C-AMS measurement, because they produce reliable ion current (C^-) with low sample to sample contamination (*7*). Furthermore, graphite or graphite-like materials can be handled at facilities which have ambient level ^{14}C-AMS, because it lacked vapor pressure (*8*). For those reasons,

several sample preparation protocols for accurate and precise ^{14}C-AMS measurement have been reported.

Graphitization methods based on the Boudouard's reactions (*9*) have been widely used for all ^{14}C-AMS applications including biological/biomedical applications. Although various elements in the periodic table have catalytic activity for graphitization (Figure 1c), Fe or Co was mostly used as a catalyst. Table 1 summarized the differences of four graphitization methods that we have used for ^{14}C food component and nutrient studies (*10-13*). Most forward graphitization reactions (Table 1) were thermodynamically preferred in the range of 450 – 650 °C. Detailed graphitization protocol previously reported (*11*) and instruction video was also available in http://nutrition.ucdavis.edu/faculty/clifford.html. Recently, we further optimized our graphitization method for accurate and precise HT-^{14}C-AMS measurement.

Table 1. Summary of differences of chemical reactions during catalytic graphitization methods. Some side chemical reactions were occurred at low temperature, high H_2 concentration, or both.[†]

Our applications	CO_2 reduction (graphitization) conditions[‡]	Graphitization chemical reactions
^{14}C-Folic acid ^{14}C-Vitamin E	Flame-sealed tube system Reductant: ≥ 40 mg Zn + 10 – 40 mg TiH_2 Catalyst: Proper amounts of Co Reduction : 500 °C, 3 h +550 °C, 2 h (*12*)	$TiH_2 \rightarrow Ti + H_2$ $CO_2 + H_2 \rightarrow CO + H_2O$ $H_2O + Zn \rightarrow H_2 + ZnO$ $CO_2 + Zn \rightarrow CO + ZnO$ $CO + H_2 \rightarrow C + H_2O$ $2CO \rightarrow C + CO_2$
^{14}C-Vitamin E	Septa-sealed vial system, Reductant: 75 – 150 mg Zn Catalyst: 2 – 3 mg of -400 mesh Fe Reduction : 500 °C, ≈ 4 h (*13*)	$Zn + H_2O \rightarrow H_2 + ZnO$ $Zn + CO_2 \rightarrow CO + ZnO$ $CO_2 + H_2 \rightarrow CO + H_2O$ $CO + H_2 \rightarrow C + H2O$ $2CO \rightarrow C + CO_2$ $CO_2 + 2H_2 \rightarrow C + 2H_2O$
^{14}C-Vitamin E ^{14}C-Lutein	Septa-sealed vial system Reductant: ≈ 100 mg Zn Catalyst: ≈ 10 mg of -400 mesh Fe Reduction : 525 °C, 6 h (*11*)	
Current our optimized method	Septa-sealed vial system Reductant: 100 mg Zn Catalyst: 5 mg of -400 mesh Fe Reduction : 500 °C, 3 h (*10*)	

[†] $CO + 3H_2 \rightarrow CH_4 + H_2O$ and $2CO + 2H_2 \rightarrow CH_4 + CO_2$

[‡] Reduction conditions were optimized for one milligram sized carbon samples

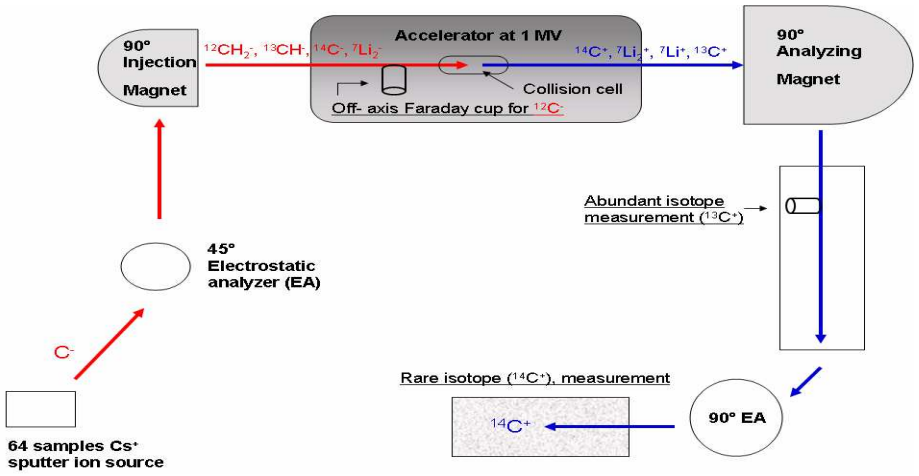

Figure. 2. One million voltage (1 MV) AMS for biological/biomedical applications at the CAMS LLNL (*14*).

Accelerator Mass Spectrometry (AMS). AMS generally consists of ionizer using Cs, low to high energy magnetic mass analyzers, electrostatic analyzers, accelerator (0.2 – 10 MV), Faraday cups (for ^{12}C, ^{13}C measurement), and (gas ionization or solid) detector for ^{14}C (*3, 7, 15*). Figure 2 was a schematic of 1 MV AMS for biological/biomedical applications at the CAMS LLNL (*14*).

Graphitized samples in the AMS target holder were bombarded with cesium sputter (Cs^+) and ionized to produce a negatively charged carbon ion beam current (C^-) without ^{14}N isobar interference (*16-17*). Gas sample (CO_2 after combustion step) was an alternative type for ^{14}C-AMS measurement. The CO_2 transferred (on-line) to an AMS system, then can directly produce C^- currents using Cs^+ sputter without graphitization, so that it could save labor time and cost for graphitization. Direct ionization system of CO_2 was usually coupled with GC, HPLC, or other conventional MS (*18*). Although gas sample enabled the ^{14}C-AMS measurement, it had produced lower ion currents and high sample to sample contamination (*7, 19-20*). A C^- current entered an injection magnet where C^- current was separated by mass to charge ratio (*m/z*), and ^{12}C, ^{13}C, and ^{14}C ions passed through separately. A C^- current was continually passed into the

accelerator where negatively charged ions were attracted to the positive terminal. Most AMS systems employed an electrostatic tandem accelerator with 0.2 – 10 MV (*3, 21-22*). Biological/biomedical applications were usually performed with ≤ 1 MV accelerator (*3, 14-15, 23*). During the accelerator step, C^- was collided with argon gas or a thin carbon foil in a collision cell called stripping where C^- was changed to positively charged carbon ions (C^+ to C^{4+}) depending on accelerator energy. Furthermore, molecular isobars of ^{14}C such as $^{12}CH_2^-$, $^{12}CD^-$, or $^{13}CH_1^-$ were destroyed, because their positively charged forms were unstable. Stripping with thin carbon foil had stronger ion beam transmission and higher charge state than those with argon gas. Thin carbon foil should be changed with time due to its limited life-time and radiation damage. Especially, heavy ions were necessary for more frequently changing thin carbon foil compared to lighter ions. Stripping with argon gas was usually used in modern tandem accelerator (especially for lighter ions) due to better ion beam transmission stability (*7*). Atomic C^+ was repelled and exited at the end of positive terminal in accelerator. Atomic C^+ such as $^{13}C^+$, and $^{14}C^+$ were further separated with their *m/z* in high energy magnetic analyzer. A $^{13}C^+$ was measured in a Faraday cup after the accelerator step. A $^{14}C^+$ was further focused/stabilized using quadruple/electrostatic cylindrical analyzer, and then was measured in the gas ionization or solid detector. Measured ratio of $^{14}C/^{12}C$ or $^{14}C/^{13}C$ in samples was defined as "Modern or Fraction Modern (F_m)" was standardized using oxalic acids (NIST SRM 4990B, 4990C) or sucrose (IAEA-C6) (*24-25*).

Supporting Information, Table2.

Radiation exposure calculation sheets from ^{14}C-nutrients were available to the other attached Excel file.

Table 3. Common (natural and human made) radiation source/exposure and annual radiation exposure limits.[a]

Source	Average Radiation Dose Per View
One coast-to coast airline flight (≈4 hr)	2 mrem (0.02 mSv)[b]
Normal chest examination	20 mrem (0.2 mSv)
Natural background radiation in the U.S.	300mrem (3mSv)/yr
Normal dental examination	20 mrem (0.2 mSv)
Rib cage examination	140 mrem (1.4 mSv)
Gall bladder examination	170 mrem (1.7 mSv)
Barium enema examination	500 mrem (5.0 mSv)
Pelvic examination	600 mrem (6.0 mSv)

Specimen	Radiation exposure limit (mrem/yr)[a]
Whole body	5,000
Extremities	50,000
Fetus	500

[a] Adapted from the National Council Radiological Protection (NCRP) Report No. 94.

[b] Sv = Joule/kg = 0.01 rem

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